Plant Physiol. (1991) 97, 693-698
0032-0889/91/97/0693/06/$01.00/0

Received for publication February 11, 1991
Accepted June 13, 1991

Elicitor-Inducible 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity Is Required for Sesquiterpene Accumulation in Tobacco Cell Suspension Cultures[1]

Joseph Chappell*, Carol VonLanken, and Urs Vögeli[2]

Plant Physiology/Biochemistry Program, Agronomy Department, University of Kentucky, Lexington, Kentucky 40546-0091

ABSTRACT

Addition of cell wall fragments from *Phytophthora* species or cellulase from *Trichoderma viride*, but not pectolyase from *Aspergillus japonicus*, to tobacco (*Nicotiana tabacum*) cell suspension cultures induced the accumulation of the extracellular sesquiterpenoid capsidiol. Pulse-labeling experiments with [^{14}C]acetate and [^{3}H]mevalonate suggested that enzymatic steps preceding mevalonate were limiting capsidiol biosynthesis in the pectolyase-treated cell cultures. Treatment of the cell cultures with either *Phytophthora* cell wall fragments or cellulase induced 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and sesquiterpene cyclase activities, enzymes of the sesquiterpene biosynthetic pathway, and phenylalanine ammonia lyase activity, an enzyme of the general phenylpropanoid pathway. Pectolyase treatment induced sesquiterpene cyclase and phenylalanine ammonia lyase activities, but not HMGR activity. These results corroborate the importance of inducible HMGR enzyme activity for sesquiterpene accumulation.

The defensive response of plants to pathogen attack includes the production of antimicrobial compounds known as phytoalexins (2). This response results from recognition of the pathogen and transduction of that information into an activation of the phytoalexin biosynthetic pathway (11). Although the recognition phenomenon remains a complex process not fully understood, oligosaccharides released from fungal and plant cell walls are often sufficient to elicit phytoalexin biosynthesis (6). Two particularly active classes of oligosaccharide elicitors, glucans and polygalacturonides, have been chemically characterized for optimal polymer length and bond configuration (6). The best characterized glucan elicitors were initially derived from autoclaving (1) or acid hydrolysis (18) of *Phytophthora megasperma* cell walls, and elicited glyceollin accumulation when applied to mechanically wounded soybean cotyledons and hypocotyls. Polygalacturonide polymers released from citrus pectin and plant cell walls by chemical and enzymatic hydrolysis have also been characterized for their ability to elicit phytoalexin biosynthesis by soybean cotyledons (8, 12, 17) and castor bean hypocotyls (4, 15), as well as proteinase inhibitor(s) in wounded tomato and potato leaves (23). More recently, synergisms of glucan and polygalacturonide elicitors for phytoalexin biosynthesis in soybean (9) and parsley (10) have been reported. In addition to these well characterized glucan and polygalacturonide elicitors, other biotic and abiotic elicitors are known. These include fungal enzymes capable of digesting plant cell walls (cellulase, pectinase, pectolyase, polygalacturonase) (7, 15, 21), fatty acids (arachidonic and eicosapentaenoic acids) (3), and heavy metals (Hg) (13).

We (5) previously described the induced synthesis and secretion of sesquiterpene-type phytoalexins by tobacco cell suspension cultures responding to fungal elicitor preparations from *P. parasitica*. The elicitor-induced synthesis of sesquiterpenoids was subsequently correlated with the induction of two enzymes of the sesquiterpenoid biosynthetic pathway, HMGR[3] (EC 1.1.1.34) and sesquiterpene cyclase (22). The induction of sesquiterpenoid accumulation was also correlated with a suppression of sterol biosynthesis, an isoprenoid branch pathway that could be competing with sesquiterpene biosynthesis for farnesyl diphosphate, the last common intermediate between these two pathways. The suppression of sterol biosynthesis was correlated with a suppression of squalene synthetase (EC 2.5.1.21), the first committed enzyme for this biosynthetic pathway (22).

Because we are intent on generating large amounts of elicitor-induced tobacco cells for the purposes of purifying the enzymes of the sesquiterpenoid biosynthetic pathway, we initiated a screening for the most potent and easily obtainable elicitors. In preliminary experiments, we observed that the tobacco cell suspension cultures responded differentially to commercially available cellulase (*Trichoderma viride*) and pectolyase (*Aspergillus japonicus*). Both elicitors induced a color change in the cultures within 2 h of their addition, but only media from the cultures treated with cellulase contained the expected sesquiterpenoids. Threlfall and Whitehead (21) recently reported a similar finding, that cellulase (*T. viride*)

[1] This work was supported by the Kentucky Agricultural Experiment Station, the R.J. Reynolds Corporation, a Swiss National Science Foundation fellowship (to U.V.), a cooperative grant from the U.S. Department of Agriculture, and the National Science Foundation. This is journal paper 89-3-158 of the Kentucky Agricultural Experiment Station.

[2] Present address: Dr. R. Maag Ltd., Agrochemicals, CH-8157 Dielsdorf, Switzerland.

[3] Abbreviations: HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; PAL, phenylalanine ammonia lyase; Pp elicitor, elicitor prepared by hydrolysis of the cell wall of *P. parasitica*.

but not pectinase (*A. niger*) was an active elicitor of bicyclic sesquiterpenoids in pepper and tobacco. Surprised by the obvious browning of the pectolyase-treated tobacco cell cultures without production of extracellular sesquiterpenoids, we examined whether select enzymes of the sesquiterpenoid biosynthetic pathway were induced.

MATERIALS AND METHODS

Cell Cultures and Elicitor Treatments

Cell suspension cultures of tobacco (*Nicotiana tabacum* L. cv KY 14) were maintained in Murashige-Skoog medium, subcultured weekly, and their growth monitored by measuring the increase in fresh weight. Cultures in the rapid phase of growth (approximately 3 d after subculturing, fresh weight doubling every 2 d) were used in all the experiments presented. Elicitor treatments were initiated by the addition of *Phytophthora parasitica* cell wall hydrolysate, cellulase (*Trichoderma viride,* Karlan Chemical Corp., Torrance, CA), pectolyase (*Aspergillus japonicus,* Karlan), or other putative elicitors at the indicated concentrations per mL of tobacco cell culture. Depending on the chemical nature of an elicitor, whether protein or carbohydrate, the concentration of an elicitor is presented as μg of elicitor protein powder (as supplied by the manufacturer) or carbohydrate equivalents. Fungal cell wall hydrolysates were prepared from *Phytophthora* species according to Ayers *et al.* (1) and were quantified as anthrone-positive carbohydrate with glucose as a standard. Cellulase and pectolyase powders as supplied by the manufacturer were dissolved in Murashige-Skoog medium and filter-sterilized before use. Arachidonic acid was purchased from Sigma and prepared according to Bostock *et al.* (3). Pectin and polygalacturonic acid hydrolysates were kindly provided by K. Davis and P. Albersheim, University of Georgia.

Sesquiterpenoid Determinations and *in Vivo* Labeling

Sesquiterpenoids were extracted from the cell culture media by a chloroform partitioning method modified from that of Henfling and Kuć (14). The sesquiterpenoid capsidiol was quantified by GC (5).

For *in vivo* labeling experiments, 2 μCi of RS-[5-^{3}H]mevalonate (27 Ci/mmol, New England Nuclear) or 0.2 μCi of [2-^{14}C]acetate (54 Ci/mmol, New England Nuclear) were added to the cell cultures 12 h after initiation of the respective experimental treatments. After 60 (acetate) or 90 min (mevalonate) of further incubation, the cells and medium were analyzed for radioactivity incorporated into sterols and extracellular capsidiol as previously described (22).

Enzyme Assays

Assays for squalene synthetase and sesquiterpene cyclase have been previously described (22). Total HMGR activity was assayed in a microsomal fraction (100,000 *g*) generated by centrifugation of the initial 300*g* supernatant (5). No attempt was made to differentiate between putative HMGR isozymes located in different intracellular compartments, such as mitochondrial and different ER forms. PAL (EC 4.3.1.5) activity was measured according to published methods (24). Cellular extracts were prepared by homogenizing plant material in 1 volume of 10 mM Hepes, pH 7.5, 5 mM EDTA, 5 mM β-mercaptoethanol with a small amount of polyvinylpolypyrrolidone, filtering the homogenate, and centrifuging the filtrate at 10,000*g* for 20 min. The entire extraction procedure was carried out at 4°C. Aliquots of the 10,000*g* supernatant were subsequently incubated in 100 mM borate buffer, pH 8.8, containing 10 mM phenylalanine at 30°C, and the reaction was monitored for cinnamate production by absorbance at 290 nm. PAL enzyme activity is expressed as nmol of cinnamate formed, calculated using a molar extinction coefficient of 10,000. Protein concentrations were determined as previously described (5).

All the experiments reported have been reproduced three times with similar results. Each data point represents the average of duplicate determinations, with an experimental error of 10 to 20%. However, it is important to note that, although the absolute values between experiments showed much more variation (up to twofold), the relative trends remained the same.

RESULTS

Efficacy of Different Elicitors on the Induction of Sesquiterpenoid Accumulation

Elicitor activity was assessed according to the accumulation of capsidiol, a bicyclic sesquiterpenoid, in the medium of tobacco cell suspension cultures 10 to 12 h after the addition of a putative elicitor. Cell wall hydrolysates of *P. parasitica, P. infestans,* or *P. megasperma* elicited capsidiol accumulation to the same extent (2.5–50 μg of capsidiol/g fresh weight) when added to tobacco cell suspension cultures in the range of 1 to 400 μg Glc equivalents/mL of cell culture (for example, see Table I). Arachidonic acid at concentrations of 0.1 to 10 μg/mL of cell culture did not induce capsidiol production, nor did hydrolysates of pectin (0.4–40 μg dry weight/mL of cell culture) or polygalacturonic acid (0.33–30 μg dry weight/mL of cell culture). However, with the exception of arachidonic acid treatment, all the elicitors tested induced a distinct color change of the cultures, from a light yellow to an orange-brown. Color changes were usually visible within 2 h of elicitor addition to the cell cultures. The Pp elicitor, the causal agent of black shank disease in tobacco (16), was used throughout these studies for comparison to other elicitors.

A more detailed comparison of the Pp elicitor to cellulase from *T. viride* and pectolyase from *A. japonicus* is presented in Table I. All three elicitors induced browning of the cell cultures, but only the Pp elicitor and cellulase induced capsidiol accumulation. Cell cultures incubated with an optimal concentration of cellulase (0.1 μg/mL of cell culture) produced 1.5 to 2 times more capsidiol than those cultures receiving optimal amounts of the Pp elicitor (30 μg Glc equivalents/mL). Cellulases and pectin-degrading enzymes from other fungi were also examined for their abilities to induce sesquiterpenoid accumulation in tobacco cell suspension cultures. Cell cultures incubated with cellulase from *T. viride* accumulated 10 to 100 times more capsidiol than cultures treated with an equal amount of cellulase from *A. niger,* and no capsidiol accumulated in cultures treated with

Table I. *Comparison of P. parasitica Cell Wall Hydrolysate, Cellulase from T. viride, and Pectolyase from A. japonicus on the Elicitation of Sesquiterpene Accumulation by Tobacco Cell Suspension Cultures*

Elicitors were added to the cell cultures at the indicated concentrations, and the level of extracellular capsidiol was determined by GC after an additional 10 h incubation. Browning was visually assessed relative to control cell cultures 2 and 10 h after elicitor addition.

Elicitor	Elicitor Concentration	Capsidiol	Browning
		µg/g fr wt	
None (control)		0	−
Pp cell walls	0.3 µg Glc equivalents/mL	1.7	+
	3	8.9	+
	15	24.8	+
	30	45.4	+
	75	39.0	+
Cellulase	0.01 µg/mL	2.6	+
	0.05	59.2	+
	0.1	71.2	+
	0.5	47.0	+
	1.0	40.8	+
Pectolyase	0.1 ng/mL	0	−
	1.0	0	+
	10.0	0	+
	100.0	0	+
	1.0 µg/mL	0	+
	10.0	0	+
	100.0	0	+

pectinase from *Aspergillus* species or macerase from *Rhizopus* species

To discern whether the cell culture's differential response to pectolyase and cellulase was a specific or a more general stress-related response, it was desirable to measure the inducibility of an enzyme of sesquiterpenoid metabolism relative to another elicitor-inducible enzyme unrelated to sesquiterpenoid metabolism. Sesquiterpene cyclase, the first committed step of sesquiterpene biosynthesis, and PAL, the first committed step of the general phenylpropanoid pathway, were chosen for this purpose. PAL activity was induced to the same maximal activity level in a dose-dependent manner by the Pp elicitor, pectolyase, and cellulase (Fig. 1A). The induction time courses for PAL activity were similar for all three elicitors, with maximal PAL activity occurring 12 h after elicitor addition to the cell cultures (data not shown). For comparison, the dose-dependent induction of sesquiterpene cyclase activity by Pp elicitor, pectolyase, and cellulase is shown in Fig 1B. Ranked on a relative scale and using the optimal concentration of the three elicitors tested, cyclase activity was induced 100% in cellulase-treated cells, 70 to 80% in Pp elicitor-treated cultures, and approximately 20% in pectolyase-treated cells. The induction time courses for cyclase activity were similar for all three elicitors (data not shown). Either heating the cellulase and pectolyase elicitors in a boiling water bath for 5 min, acidification of the elicitors to 3 N HCl followed by neutralization with Tris buffer, or treatment with proteinase K destroyed their abilities to induce PAL or cyclase enzyme activities. Similar treatment of the Pp elicitor had no effect on its elicitation of PAL and cyclase activities.

Comparison of Elicitors on Induction of Sesquiterpenoid Biosynthesis and Suppression of Sterol Biosynthesis

Pulse-labeling experiments with [^{14}C]acetate and [^{3}H]mevalonate have previously been used to demonstrate that the relative *in vivo* synthesis rates of sterols is suppressed coordinately with an induction in the synthesis rate of sesquiterpenoids in cell cultures incubated with Pp elicitor (22). Similar experiments were performed to determine if cellulase and pectolyase treatments had similar effects. Twelve hours after initiation of the respective elicitor treatments, radiolabeled

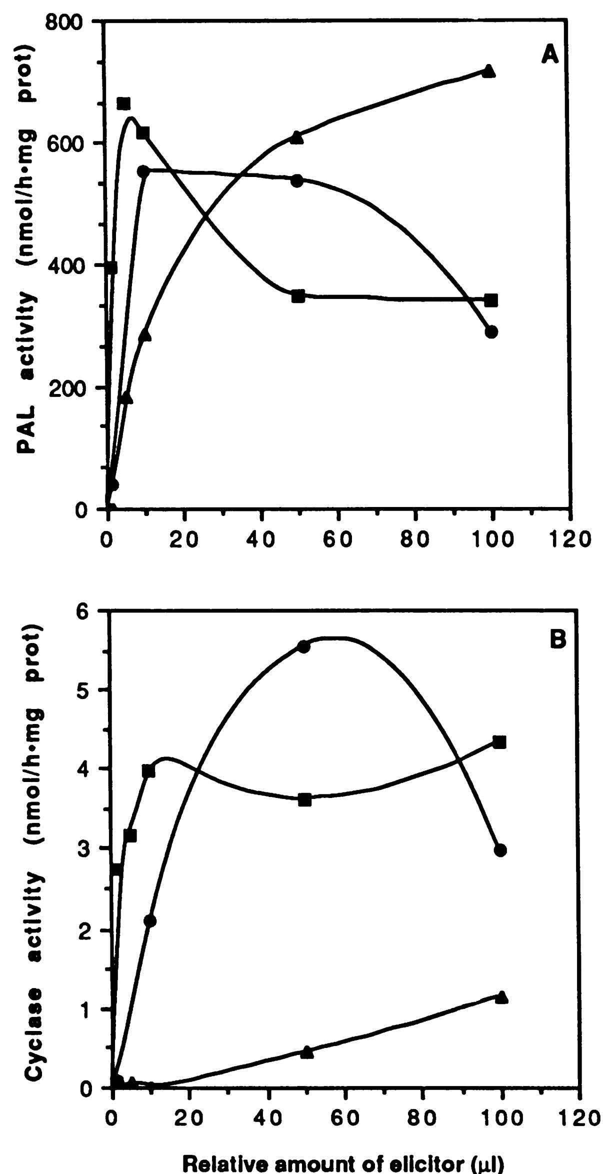

Figure 1. Dosage-dependent induction of PAL (A) and sesquiterpene cyclase (B) enzyme activities in tobacco cell suspension cultures treated with *P. parasitica* elicitor, cellulase, or pectolyase. Cell cultures (10 mL) were incubated for 10 h with the indicated amounts (µL) of Pp elicitor (3 µg Glc equivalents/µL) (●), cellulase (0.1 µg/µL)(■), or pectolyase (0.1 µg/µL)(▲) before measuring the indicated enzyme activities in cellular extracts.

Table II. *Comparison of P. parasitica Cell Wall Hydrolysate, Cellulase, and Pectolyase on the Incorporation of Radioactive Acetate and Mevalonate into Sterols and Extracellular Capsidiol by Tobacco Cell Suspension Cultures*

Cell cultures treated with 6 μg Glc equivalents/mL of Pp cell wall hydrolysate, 0.1 μg/mL of cellulase, or 1 μg/mL of pectolyase for 12 h were pulse-labeled with [^{14}C]acetate (400,000 dpm) or [^{3}H]mevalonate (4,000,000 dpm) for 60 and 90 min, respectively, before the radioactivity incorporated into cellular sterols and extracellular capsidiol was determined.

Treatment	Incorporation of Precursor			
	[^{14}C]Acetate		[^{3}H]Mevalonate	
	Sterols	Capsidiol	Sterols	Capsidiol
	dpm			
None	29,300 (14.3)[b]	0[a]	111,075 (14.4)	0
Pp cell walls	4,860 (4.6)	2,575 (2.4)	21,150 (1.9)	24,820 (2.3)
Cellulase	3,110 (3.3)	4,285 (4.6)	11,775 (1.2)	61,545 (6.1)
Pectolyase	4,330 (3.3)	580 (0.4)	28,235 (2.7)	19,780 (2)

[a] Background radioactivity has been subtracted from the values shown and was 50 and 100 dpm for the ^{14}C and ^{3}H control samples, respectively. [b] Numbers in parentheses refer to the radioactivity incorporated into that fraction as a percentage of that incorporated into the total cellular/extracellular chloroform-soluble components. This was to correct for any differential uptake of the radiolabel by the cell cultures under the different treatment regimens (22).

acetate or mevalonate was added to the cultures for a further incubation of 60 (acetate) or 90 min (mevalonate). The incorporation of each precursor into sterols and extracellular capsidiol, as well as other constituents, was determined (Table II). Of the total radioactivity incorporated into lipophilic constituents, control cells incorporated approximately 14% of either the [^{14}C]acetate or [^{3}H]mevalonate into sterols and nothing into extracellular capsidiol. Cultures incubated with Pp elicitor showed a three- and sevenfold decrease in the amount of [^{14}C]acetate and [^{3}H]mevalonate, respectively, incorporated into sterols, concomitant with a redistribution of more than 2% of the radioactivity incorporated into extracellular capsidiol. Cellulase-treated cell cultures showed an almost identical pattern of radiolabel incorporation with the exception of a two- to threefold enhancement of the [^{3}H] mevalonate incorporated into extracellular capsidiol. Pectolyase-treated cell cultures showed the same characteristic decline of [^{14}C]acetate or [^{3}H]mevalonate incorporation into sterols as the other two elicitors. However, incorporation of [^{14}C]acetate into extracellular capsidiol was negligible, whereas incorporation of [^{3}H]mevalonate was similar to that seen in the Pp elicitor-treated cell cultures.

The lack of [^{14}C]acetate incorporation into extracellular capsidiol by pectolyase-treated cell cultures was expected, because these cultures accumulated very little capsidiol. The incorporation of [^{3}H]mevalonate into capsidiol was, however, surprising. A possible explanation for this differential incorporation of [^{14}C]acetate and [^{3}H]mevalonate into extracellular sesquiterpenoids would be that enzymes of the isoprenoid biosynthetic pathway leading to mevalonate were limiting capsidiol biosynthesis in pectolyase-treated cultures, and the sesquiterpene biosynthetic enzymes subsequent to mevalonate were sufficiently induced. Alternatively, the labeling studies could represent a differential ability of the acetate and mevalonate to enter into the sesquiterpenoid biosynthetic pathway in the differently elicitor-treated cell cultures. To distinguish between these possibilities, enzymes of the sesquiterpenoid and sterol biosynthetic pathway were measured (22).

The efficacy of various elicitors to induce PAL, HMGR, and sesquiterpene cyclase enzyme activities, as well as their efficacy in suppressing squalene synthetase, are compared in Table III. As mentioned earlier, all three elicitors effectively induced PAL activity, but pectolyase treatment only partially induced sesquiterpene cyclase activity. Squalene synthetase activity measured 12 h after initiation of the elicitor treatments was suppressed greater than 65% by the various elicitors. The HMGR activity measured in cell cultures incubated with Pp elicitor and cellulase for 6 h was approximately 17- and 29-fold greater, respectively, than the level found in control cell cultures. Incubation of the cell cultures with pectolyase did not induce HMGR enzyme activity significantly (Fig. 2).

DISCUSSION

A number of reports have attempted to correlate changes of HMGR enzyme activity with the induced synthesis of particular isoprenoids. Suzuki *et al.* (20) correlated a transient induction of HMGR activity with the accumulation of ipomeamarone, a furanosesquiterpenoid, in sweet potato tissue infected with *Ceratocystis fimbriata.* Stermer and Bostock (19), likewise, have shown a transient induction of HMGR activity in potato discs stimulated to produce sesquiterpenoids by application of arachidonic acid. We also previously correlated the induction of HMGR activity in elicitor-treated tobacco cell suspension cultures with sesquiterpenoid accumulation (5). Changes in the *in vivo* synthesis rate of the extracellular capsidiol, measured by pulse-labeling the cell cultures

Table III. *Effects of P. parasitica Cell Wall Hydrolysate, Cellulase, and Pectolyase on PAL, Sesquiterpene Cyclase (SC), Squalene Synthetase (SS), and HMGR Enzyme Activities in Tobacco Cell Suspension Cultures*

Cell cultures were incubated with 5 to 6 μg Glc equivalents/mL Pp cell wall hydrolysate, 0.1 μg/mL cellulase, or 1 μg/mL pectolyase for 6 h (HMGR determination) and 12 h (PAL, SC, SS determinations) before measuring enzyme activities.

Treatment	Enzyme Activities[a]			
	PAL	SC	SS	HMGR
	nmol/mg protein·h			
None	75	0.0	6.7	2.7
Pp cell walls	550	6.8	1.2	46
Cellulase	690	7.5	2.1	78.5
Pectolyase	760	2.8	1.9	3.7

[a] Enzyme activities measured in separate experiments.

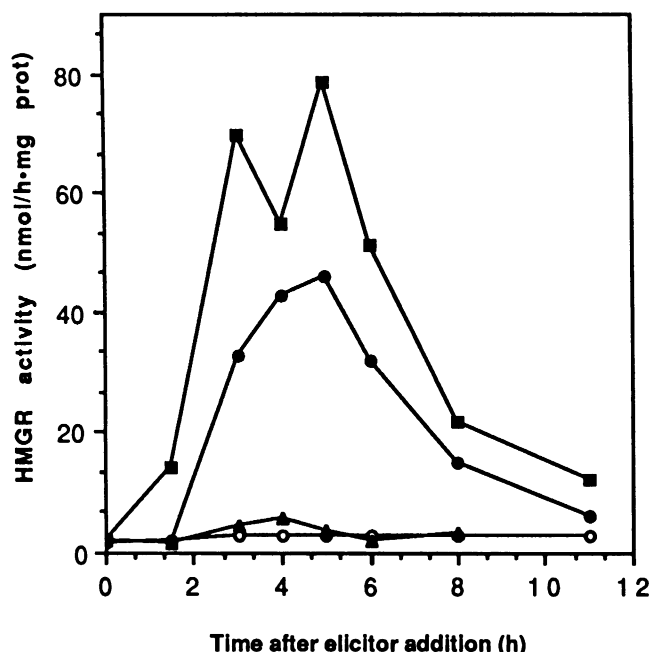

Figure 2. Induction time course of HMGR enzyme activity in tobacco cell suspension cultures treated with *P. parasitica* elicitor, cellulase, or pectolyase. Enzyme activity was measured in extracts prepared from control cultures (○) and cultures receiving Pp elicitor (6.9 μg Glc equiv/mL)(●), cellulase (0.1 μg/mL)(■), or pectolyase (1.0 μg/mL)(▲).

with [^{14}C]acetate, were similar to the transient induction time course of HMGR enzyme activity, and the elicitor-induced accumulation of capsidiol was sensitive to mevinolin, a potent competitive inhibitor of HMGR enzyme activity (5). Yet, those studies were tempered by our observations that [^{3}H] mevalonate incorporation into sesquiterpenoids was also transiently induced and partially inhibited by mevinolin. The implications of these latter results were that reactions distal to HMGR were also regulated, possibly limiting capsidiol biosynthesis, and, because mevinolin was not as specific an inhibitor in plants as in animals, it could not be used to unequivocally demonstrate the necessity of HMGR activity for capsidiol biosynthesis.

The importance of the inducible HMGR activity for elicitor-stimulated sesquiterpenoid biosynthesis was further corroborated by the current results. Tobacco cell suspension cultures incubated with pectolyase did not exhibit a transient induction of HMGR activity, nor did they accumulate extracellular sesquiterpenoids. This was in spite of the fact that all the necessary biosynthetic machinery beyond HMGR was apparently induced, as well as other responses such as culture browning and induction of PAL. Hence, pectolyase treatment was rather conspicuous because only HMGR was not induced.

In comparison to Pp elicitor-treated cell cultures, cell cultures incubated with pectolyase incorporated [^{3}H]mevalonate into capsidiol equally well. This was surprising, because pectolyase treatment only induced sesquiterpene cyclase 20 to 50% of that observed with the other elicitors, and suggested that some other catalytic step between mevalonate and farnesyl diphosphate must be limiting the incorporation rate of exogenous [^{3}H]mevallnate into capsidiol. However, the [^{3}H] mevalonate incorporation data are also confounded by not knowing how the endogenous mevalonate pool size is changing. Because of reduced HMGR activity, the specific activity of the endogenous mevalonate pool would be greater in pectolyase-treated cultures fed [^{3}H]mevalonate. This would result in a greater amount of radioactivity being converted to capsidiol, thus giving the apparent impression that the catalytic steps beyond HMGR were not limiting. Despite this caveat, the lack of an induction of HMGR activity in pectolyase-treated cell cultures was obviously a limiting factor for capsidiol production.

These conclusions extend our previous finding that mevinolin, a competitive inhibitor of HMGR, inhibited capsidiol accumulation in elicitor-treated cell cultures (5). In those earlier studies, the mevinolin inhibition could not possibly discriminate between putative HMGR isozymes, such as those found in control cells *versus* elicitor-treated cells. In contrast, the current results clearly demonstrate that the HMGR activity found in control cell cultures is insufficient for sesquiterpene biosynthesis. And this obviously suggests that the induced and constitutive forms of HMGR are dedicated to the synthesis of different isoprenoid products with little ability of one enzyme to complement the loss of the other. A more direct test of this interpretation must await the generation of genetic mutations that alter the expression of the respective HMGR enzyme activities and measurement of the levels of various isoprenoids, such as sesquiterpenes in elicitor-treated cells.

LITERATURE CITED

1. **Ayers AR, Ebel J, Valent B, Albersheim P** (1976) Host-pathogen interactions. X. Fractionation and biological activity of an elicitor isolated from the mycelial walls of *Phytophthora megasperma* var. sojae. Plant Physiol **57:** 760–765
2. **Bell AA** (1981) Biochemical mechanisms of disease resistance. Annu Rev Plant Physiol **32:** 21–81
3. **Bostock RM, Schaeffer DA, Hammerschmidt R** (1986) Comparison of elicitor activities of arachidonic acid, fatty acids and glucans from *Phytophthora infestans* in hypersensitivity expression in potato tuber. Physiol Mol Plant Pathol **29:** 349–360
4. **Bruce RJ, West CA** (1982) Elicitation of casbene synthetase activity in castor bean. The role of pectic fragments of the plant cell wall in elicitation by a fungal endopolygalacturonase. Plant Physiol **69:** 1181–1188
5. **Chappell J, Nable R** (1987) Induction of sesquiterpenoid biosynthesis in tobacco cell suspension cultures by fungal elicitor. Plant Physiol **85:** 469–473
6. **Darvill AG, Albersheim P** (1984) Phytoalexins and their elicitors-A defense against microbial infection in plants. Annu Rev Plant Physiol **35:** 243–275
7. **Davis KR, Lyon GD, Darvill AG, Albersheim P** (1984) Host-pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol **74:** 52–60
8. **Davis KR, Darvill AG, Albersheim P, Dell A** (1986) Host-pathogen interactions. XXIX. Oligogalacturonides released from sodium polypectate by endopolygalacturonic acid lyase are elicitors of phytoalexins in soybean. Plant Physiol **80:** 568–577
9. **Davis KR, Darvill AG, Albersheim P** (1986) Host-pathogen interactions. XXXI. Several biotic and abiotic elicitors act synergistically in the induction of phytoalexin accumulation in soybean. Plant Mol Biol **6:** 23–32

10. **Davis KR, Hahlbrock K** (1987) Induction of defense responses in cultured parsley cells by plant cell wall fragments. Plant Physiol **85:** 1286–1290
11. **Dixon RA, Lamb CJ** (1990) Molecular communication in interactions between plants and microbial pathogens. Annu Rev Plant Physiol **41:** 339–367
12. **Hahn MG, Darvill AG, Albersheim P** (1981) Host-pathogen interactions. XIX. The endogenous elicitor, a fragment of a plant cell wall polysaccharide that elicits phytoalexin accumulation in soybean. Plant Physiol **68:** 1161–1169
13. **Hargraeves JA** (1979) Investigations into the mechanism of mercuric chloride stimulated phytoalexin accumulation in *Phaseolus vulgaris* and *Pisum sativum*. Physiol Plant Pathol **15:** 279–287
14. **Henfling JWDM, Kuć J** (1979) A semi-micro method for the quantitation of sesquiterpenoid stress metabolites in potato tube tissue. Phytopathology **69:** 609–611
15. **Lee S-C, West CA** (1981) Polygalacturonase from *Rhizopus stolonifer*, an elicitor of casbene synthetase activity in castor bean (*Ricinus communis* L.) seedlings. Plant Physiol **67:** 633–639
16. **Lucas GB** (1975) Diseases of Tobacco, Ed 3. Harold E Parker and Sons, Fuquay-Varina, NC, p 621
17. **Nothnagel EA, McNeil M, Albersheim P, Dell A** (1983) Host-pathogen interactions. XXII. A galacturonic acid oligosaccharide from plant cell walls elicits phytoalexins. Plant Physiol **71:** 916–926
18. **Sharp JK, Valent B, Albersheim P** (1984) Purification and partial characterization of a β-glucan fragment that elicits phytoalexin accumulation in soybean. J Biol Chem **259:** 11312–11320
19. **Stermer BA, Bostock RM** (1987) Involvement of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the regulation of sesquiterpenoid phytoalexin synthesis in potato. Plant Physiol **84:** 404–408
20. **Suzuki H, Oba K, Uritani I** (1975) The occurrence and some properties of 3-hydroxy-3-methylglutaryl coenzyme A reductase in sweet potato roots infected by *Ceratocystis fimbriata*. Physiol Plant Pathol **7:** 265–276
21. **Threlfall DR, Whitehead IM** (1988) Co-ordinated inhibition of squalene synthetase and induction of enzymes of sesquiterpenoid phytoalexin biosynthesis in cultures of *Nicotiana tabacum*. Phytochemistry **27:** 2567–2580
22. **Vögeli U, Chappell J** (1988) Induction of sesquiterpene cyclase and suppression of squalene synthetase activities in plant cell cultures treated with fungal elicitor. Plant Physiol **88:** 1291–1296
23. **Walker-Simmons M, Jin D, West CA, Hadwiger L, Ryan CA** (1984) Comparison of proteinase inhibitor-inducing activities and phytoalexin elicitor activities of a pure fungal endopolygalacturonase, pectic fragments, and chitosans. Plant Physiol **76:** 833–836
24. **Zimmermann A, Hahlbrock K** (1975) Light-induced changes of enzyme activities in parsley cell suspension cultures. Purification and some properties of phenylalanine ammonia-lyase. Arch Biochem Biophys **166:** 54–62